PROCEEDINGS

of the

ENTOMOLOGICAL SOCIETY

of **WASHINGTON**

A Method for Growing Legumes with and without Root Nodules for Studying Nodule-attacking *Rivellia* (Diptera: Platystomatidae)

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PROC. ENTOMOL. SOC. WASH.
111(3), 2009, pp. 759–761

NOTE

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Insects that live below ground or have below ground stages are often not considered in conventional ecological theory (Johnson et al. 2006). In terrestrial insects with subterranean larvae, larvae have limited mobility relative to adults. The "preference-performance hypothesis" states that herbivorous insects with larvae that have limited locomotive ability for finding new host-plants have strong selective pressure to oviposit on plants that will allow for larval success (Johnson et al. 2006). Thus, in systems where terrestrial insects have subterranean larvae, plant condition below ground may drive above ground oviposition choice, though few studies have examined this idea (Johnson et al. 2006).

Flies in the genus *Rivellia* Robineau-Desvoidy (Diptera: Platystomatidae) are an example of insects with subterranean, nodule-feeding larvae. They are all specialists on legumes in the subfamily Faboideae (Foote et al. 1987). Females oviposit in the soil of their host-plants and newly hatched larvae enter and consume the root nodules of their host-plant (for an overview of the development of and role played by root nodules in legume growth see Lindemann and Glover 2003). These larvae have limited mobility and must find nodules below ground after hatching on the soil surface. This creates strong selection for females to oviposit on host-plants in appropriate condition (i.e, plants with healthy nodules). For this reason, flies in the genus *Rivellia* are good candidates for testing whether below ground plant condition affects above ground ovipositional behavior. Koethe and Van Duyn (1984) showed that the larvae of *R. quadrifasciata* (Macquart) had an 89% survival rate on nodulating soybean plants compared to 9% survival on nodule-free plants, so a selective advantage clearly exists for oviposition on nodulating plants. Oviposition tests using species of *Rivellia* will require the ability to see how gravid females respond to plants with and without root nodules. In this note, we describe a successful method for growing two legumes, both with and without root nodules, that may be attacked by species of *Rivellia*. This growing method will be useful for examining the ovipositional response of these terrestrial insects with subterranean larvae that attack legume root nodules.

The legume growing methods presented here should be useful to anyone studying host-plant choice in legume pest (and non-pest) systems with subterranean larvae. These methods should work for any nodule-producing legume species and thus may be applicable to many systems beyond Rivellia, such as the weevil *Sitona lineatus* L. (Coleoptera: Curculionidae) that attacks peas and beans (George 1962).

Two legume species were used for this experiment: *Desmodium paniculatum* and *D. canadense*, as at least one *Rivellia* species (*R. steyskali* Namba and probably *R. quadrifasciata*) utilizes *Demodium* spp. (Foote et al. 1987). Seeds for both plant species were obtained from the Prairie Moon Nursery (Winona, MN, USA) and germinated in Fafard® Superfine Germinating Mix. All germinating mix and soil was moistened and autoclaved for 20 minutes prior to use to eliminate all *Rhizobia*, the bacteria re-

* Accepted by Michael W. Gates

sponsible for triggering nodule growth in legumes (Lindemann and Glover 2003). About two weeks after germination, the seedlings were transferred to 10.5 cm × 10.5 cm pots filled with autoclaved Fafard® 1-P soil. Forty plants of each species were transplanted singly into pots for a total of 80 plants. Half of these pots for each species were inoculated with *Rhizobia* by liberally applying Burpee® Booster for Peas and Beans (which contains many different *Rhizobium* spp.) to the soil, creating nodule and non-nodule treatments. Because root nodules help fix nitrogen from the air for use by the plant, we fertilized our non-nodule treatment plants with a general 20-20-20 (N-P-K) fertilizer to ensure enough nitrogen was present for proper plant development. We waited five days before fertilizing any of the seedlings to allow them to get established in their respective pots. After a few weeks of only fertilizing the nodule-free plants, fertilizer was applied to the *Rhizobia*-treated plants, as they were not growing vigorously and this kind of fertilizer does not negatively affect root nodules in legumes (Lindemann and Glover 2003). All plants were grown in the Kent State University research greenhouse under natural light conditions between May and August and were watered daily. The *Rhizobia*-inoculated and *Rhizobia*-free plants were kept separated on a long bench in the greenhouse. About seven weeks after transplanting the seedlings into individual pots, all plants were killed and their roots examined for nodules.

This method of growing plants to obtain individuals with and without root nodules was successful. Of the 40 plants (20 from each species) in the *Rhizobia*-innoculated treatment, all 40 had nodules. Of the 40 plants in the *Rhizobia*-free treatment, only two had nodules (both *D. canadense*). The two plants with unexpected nodules likely grew the nodules via soil (*Rhizobia*) contamination from one of the *Rhizobia*-inoculated pots. We presume the contamination occurred when seedlings were knocked over within their pots during watering and propped up by the experimenter without washing hands after contacting plants or soil, or by soil insects moving between pots. These results suggest that with reasonable care and better plant separation, this method of growing plants may be a reliable way to get plants for study of female oviposition choice in *R. steyskali* or *R. quadrifasciata*. This growing method should also work for other legume species and thus for other species of *Rivellia* or other nodule-attacking insects.

Durst and Bosworth (1986) noted that nodules with a pink center are active and healthy, so when the plants were sacrificed, a plant was randomly selected and a sample of its nodules cut open. The insides of the nodules were as described by Durst and Bosworth (1986) as being healthy and active, so this method produces plants with healthy nodules. When the plants were sacrificed, the *Rhizobia*-free individuals were slightly larger, possibly due to the use of chemical fertilizer early in development. The roots were also finer and denser in the *Rhizobia*-free plants. However, based on qualitative observations, the *Rhizobia*-inoculated plants were generally greener and healthier looking.

To see how our greenhouse-grown root nodules compared to naturally grown nodules in the field, 28 nodules representing four *D. paniculatum* plants were collected in the field (all plants collected in Portage County, OH, USA) and compared to 28 nodules representing four greenhouse-raised *D. paniculatum* plants. Diameters of the widest axis on the mostly spherical nodules were measured and compared between the two populations. The nodules were not different between greenhouse and field plants (Means ± 1SE: greenhouse nodules = 1.67 ± 0.13 mm, field nodules = 1.67 ± 0.11 mm; *t*-test: $t = 0.07$; $df = 54$; $P = 0.94$). Though nodule size was similar between greenhouse and field-grown plants, it was observed that large tap roots were present in all field plants, while they were greatly reduced or absent in the greenhouse-raised plants. This could be because the small size of the pots prevented the greenhouse-raised plants from producing a large taproot, or because the taproots in the field represented growth from previous years since *Desmodium* are perennial plants.

Growing legumes with and without nodules can help answer some fundamental questions about host/oviposition choice in legume-associated insects such as *Rivellia*. Future work using these methods could include testing whether species of *Rivellia* will oviposit only on nodule-producing plants, or only on plants with large, active nodules. There are different *Rivellia* species that attack different legumes, one of which (*R. quadrifasciata*) is a pest on cultivated soybeans in the southern U.S. (Koethe and Van Duyn 1984). Gaining an understanding of basic oviposition choice by nodule-attacking insects such as *Rivellia* will not only be useful in testing hypotheses like the "preference-performance hypothesis" relative to subterranean insects, but may lead to better methods of controlling pests like *R. quadrifasciata*. Koethe and Van Duyn (1984) found that *R. quadrifasciata* larvae are more successful on nodulating soybean plants than non-nodulating plants, however they did not examine oviposition behavior relative to the two plant types. Also, the nodulating and non-nodulating plants used by Koethe and Van Duyn (1984) were different strains of soybeans. Differences between strains (other than nodule production) may have affected larval survival, so repeating their larval success experiment using the plant growing methods reported here with the same plant strains may prove useful. The ability to produce nodulating and non-nodulating plants of the same strain should open up the possibility for studies of oviposition behavior in species of *Rivellia* and other species with nodule-feeding larvae.

We thank Chris Rizzo for his advice and assistance during this experiment.

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